(2) *Health and environmental effects data.* All existing data regarding health and environmental effects of the microorganism must be reported in accordance with § 725.160.

§ 725.370 EPA review of the TME application.

General procedures for review of all submissions under this part are contained in §§ 725.28 through 725.60. In addition, the following procedures apply to EPA review of TME applications submitted under this subpart:

(a) No later than 45 days after EPA receives a TME, the Agency will either approve or deny the application.

(b) A submitter may only proceed with test marketing activities after receipt of EPA approval.

(c) In approving a TME application, EPA may impose any restrictions necessary to ensure that the microorganism will not present an unreasonable risk of injury to health and the environment as a result of test marketing.

Subpart G—General Exemptions for New Microorganisms

§ 725.400 Scope and purpose.

(a) This subpart describes exemptions from reporting under subpart D of this part, and from review under this part altogether, for manufacturing and importing of certain new microorganisms for commercial purposes.

(b) Recipient microorganisms eligible for the tiered exemption from review under this part are listed in § 725.420.

(c) Criteria for the introduced genetic material contained in the new microorganisms are described in § 725.421.

(d) Physical containment and control technologies are described in § 725.422.

(e) The conditions for the Tier I exemption are listed in § 725.424.

(f) In lieu of complying with subpart D of this part, persons using recipient microorganisms eligible for the tiered exemption may submit a Tier II exemption request. The limited reporting requirements for the Tier II exemption, including data requirements, are described in §§ 725.450 and 725.455.

(g) EPA review procedures for the Tier II exemption are set forth in § 725.470.

(h) Subparts A through C of this part apply to any submission under this subpart.

§ 725.420 Recipient microorganisms.

The following recipient microorganisms are eligible for either exemption under this subpart:

(a) *Acetobacter aceti.*
(b) *Aspergillus niger.*
(c) *Aspergillus oryzae.*
(d) *Bacillus licheniformis.*
(e) *Bacillus subtilis.*
(f) *Clostridium acetobutylicum.*
(g) *Escherichia coli* K-12.
(h) *Penicillium roqueforti.*
(i) *Saccharomyces cerevisiae.*
(j) *Saccharomyces uvarum.*

§ 725.421 Introduced genetic material.

For a new microorganism to qualify for either exemption under this subpart, introduced genetic material must meet all of the criteria listed in this section.

(a) *Limited in size.* The introduced genetic material must consist only of the following:

(1) The structural gene(s) of interest.

(2) The regulatory sequences permitting the expression of solely the gene(s) of interest.

(3) Associated nucleotide sequences needed to move genetic material, including linkers, homopolymers, adaptors, transposons, insertion sequences, and restriction enzyme sites.

(4) The nucleotide sequences needed for vector transfer.

(5) The nucleotide sequences needed for vector maintenance.

(b) *Well-characterized.* For introduced genetic material, well-characterized means that the following have been determined:

(1) The function of all of the products expressed from the structural gene(s).

(2) The function of sequences that participate in the regulation of expression of the structural gene(s).

(3) The presence or absence of associated nucleotide sequences and their associated functions, where associated nucleotide sequences are those sequences needed to move genetic material including linkers, homopolymers, adaptors, transposons, insertion sequences, and restriction enzyme sites.

(c) *Poorly mobilizable.* The ability of the introduced genetic material to be transferred and mobilized is inactivated, with a resulting frequency of transfer of less than 10^{-8} transfer events per recipient.

(d) *Free of certain sequences.* (1) The introduced genetic material must not contain a functional portion of any of the toxin-encoding sequences described in this paragraph (d).

(i) For the purposes of this section, a functional portion of a toxin-encoding sequence means any sequence which codes for a polypeptide that has one of the following effects:

(A) It directly or indirectly contributes to toxic effects in humans. Directly contributes to toxic effects in humans means those sequences encoding polypeptides that have direct toxicity to target cells. An example of a sequence which directly contributes to toxic effects in humans is one which encodes the portion of diphtheria toxin, listed in paragraph (d)(2) of this section, capable of interacting with elongation factor 2, leading to inhibition of protein synthesis in target respiratory, heart, kidney, and nerve tissues. Indirectly contributes to toxic effects in humans means a sequence whose encoded polypeptide is not directly toxic to target cells, yet still adversely affects humans. An example of a sequence which indirectly contributes to toxic effects is the sequence which encodes the portion of the botulinum toxin, listed in paragraph (d)(3) of this section, capable of blocking the release of acetylcholine from gangliosides. Botulinum toxin affects neuromuscular junctions by its blockage of acetylcholine release, leading to irreversible relaxation of muscles and respiratory arrest.

(B) It binds a toxin or toxin precursor to target human cells.

(C) It facilitates intracellular transport of a toxin in target human cells.

(ii) While these toxins are listed (with synonyms in parentheses) in paragraphs (d)(2) through (d)(7) of this section according to the source organism, it is use of the nucleotide sequences that encode the toxins that is being restricted and not the use of the source organisms. The source organisms are listed to provide specificity in identification of sequences whose use is restricted. Although similar or identical sequences may be isolated from organisms other than those listed below in paragraphs (d)(2) through (d)(7) of this section, these comparable toxin sequences, regardless of the organism from which they are derived, must not be included in the introduced genetic material.

(2) *Sequences for protein synthesis inhibitor.*

Sequence Source	Toxin Name
Corynebacterium diphtheriae & *C. ulcerans*	Diphtheria toxin
Pseudomonas aeruginosa	Exotoxin A
Shigella dysenteriae	Shigella toxin (Shiga toxin, Shigella dysenteriae type I toxin, Vero cell toxin)
Abrus precatorius, seeds	Abrin
Ricinus communis, seeds	Ricin

(3) *Sequences for neurotoxins.*

Sequence Source	Toxin Name
Clostridium botulinum	Neurotoxins A, B, C1, D, E, F, G (Botulinum toxins, botulinal toxins)
Clostridium tetani	Tetanus toxin (tetanospasmin)
Proteus mirabilis	Neurotoxin
Staphylococcus aureus	Alpha toxin (alpha lysin)
Yersinia pestis	Murine toxin
Snake toxins	
Bungarus caeruleus	Caeruleotoxin
Bungarus multicinctus	Beta-bungarotoxin (phospholipase)
Crotalus spp.	Crotoxin (phospholipase)
Dendroaspis viridis	Neurotoxin
Naja naja varieties	Neurotoxin
Notechia scutatus	Notexin (phospholipase)
Oxyuranus scutellatus	Taipoxin
Invertebrate toxins	
Chironex fleckeri	Neurotoxin
Androctnus australis	Neurotoxin
Centruroides sculpturatus	Neurotoxin

(4) *Sequences for oxygen labile cytolysins.*

Sequence Source	Toxin Name
Bacillus alve	Alveolysin
Bacillus cereus	Cereolysin
Bacillus laterosporus	Laterosporolysin
Bacillus thuringiensis	Thuringiolysin
Clostridium bifermentans	Lysin
Clostridium botulinum	Lysin
Clostridium caproicum	Lysin
Clostridium chauvoei	Delta-toxin
Clostridium histolyticum	Epsilon-toxin
Clostridium novyi	Gamma-toxin
Clostridium oedematiens	Delta-toxin
Clostridium perfringens	Theta-toxin (Perfringolysin)
Clostridium septicum	Delta-toxin
Clostridium sordellii	Lysin
Clostridium tetani	Tetanolysin
Listeria monocytogenes	Listeriolysin (A B)
Streptococcus pneumoniae	Pneumolysin
Streptococcus pyogene	Streptolysin O (SLO)

(5) *Sequences for toxins affecting membrane function.*

Sequence Source	Toxin Name
Bacillus anthracis	Edema factor (Factors I II); Lethal factor (Factors II III)
Bacillus cereus	Enterotoxin (diarrheagenic toxin, mouse lethal factor)
Bordetella pertussis	Adenylate cyclase (Heat-labile factor); Pertussigen (pertussis toxin, islet activating factor, histamine sensitizing factor, lymphocytosis promoting factor)
Clostridium botulinum	C2 toxin
Clostridium difficile	Enterotoxin (toxin A)
Clostridium perfringens	Beta-toxin; Delta-toxin
Escherichia coli & other Enterobacteriaceae spp.	Heat-labile enterotoxins (LT); Heat-stable enterotoxins (STa, ST1 subtypes ST1a ST1b; also STb, STII)
Legionella pneumophila	Cytolysin
Vibrio cholerae & Vibrio mimicus	Cholera toxin (choleragen)

(6) Sequences that affect membrane integrity.

Sequence Source	Toxin Name
Clostridium bifermentans & other *Clostridium spp*	Lecithinase
Clostridium perfringens	Alpha-toxin (phospholipase C, lecithinase); Enterotoxin
Corynebacterium pyogenes & other Corynebacterium spp.	Cytolysin (phospholipase C), Ovis toxin (sphingomyelinase D)
Staphylococcus aureus	Beta-lysin (beta toxin)

(7) *Sequences that are general cytotoxins.*

Sequence Source	Toxin Name
Adenia digitata	Modeccin
Aeromonas hydrophila	Aerolysin (beta-lysin, cytotoxic lysin)
Clostridium difficile	Cytotoxin (toxin B)
Clostridium perfringens	Beta-toxin; Epsilon-toxin; Kappa-toxin
Escherichia coli & other Enterobacteriaceae spp.	Cytotoxin (Shiga-like toxin, Vero cell toxin)
Pseudomonas aeruginosa	Proteases
Staphylococcus aureus	Gamma lysin (Gamma toxin); Enterotoxins (SEA, SEB, SEC, SED SEE); Pyrogenic exotoxins A B; Toxic shock syndrome toxins (TSST-1)
Staphylococcus aureus & Pseudomonas aeruginosa	Leucocidin (leukocidin, cytotoxin)
Streptococcus pyogenes	Streptolysin S (SLS); Erythrogenic toxins (scarlet fever toxins, pyrogenic exotoxins)
Yersinia enterocolitica	Heat-stable enterotoxins (ST)

§ 725.422 Physical containment and control technologies.

The manufacturer must meet all of the following criteria for physical containment and control technologies for any facility in which the new microorganism will be used for a Tier I exemption; these criteria also serve as guidance for a Tier II exemption.

(a) Use a structure that is designed and operated to contain the new microorganism.

(b) Control access to the structure.

(c) Provide written, published, and implemented procedures for the safety of personnel and control of hygiene.

(d) Use inactivation procedures demonstrated and documented to be effective against the new microorganism contained in liquid and solid wastes prior to disposal of the wastes. The inactivation procedures must reduce viable microbial populations by at least 6 logs in liquid and solid wastes.

(e) Use features known to be effective in minimizing viable microbial populations in aerosols and exhaust gases released from the structure, and document use of such features.

(f) Use systems for controlling dissemination of the new microorganism through other routes, and document use of such features.

(g) Have in place emergency clean-up procedures.

§ 725.424 Requirements for the Tier I exemption.

(a) *Conditions of exemption.* The manufacture or import of a new microorganism for commercial purposes is not subject to review under this part if all of the following conditions are met for all activities involving the new microorganism:

(1) The recipient microorganism is listed in and meets any requirements specified in § 725.420.

(2) The introduced genetic material meets the criteria under § 725.421.

(3) The physical containment and control technologies of any facility in which the microorganism will be manufactured, processed, or used meet the criteria under § 725.422.

(4) The manufacturer or importer submits a certification described in paragraph (b) of this section to EPA at least 10 days before commencing initial manufacture or import of a new microorganism derived from a recipient microorganism listed in § 725.420.